
INDIANA

Epidemiology

NEWSLETTER

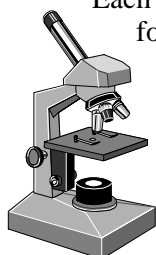


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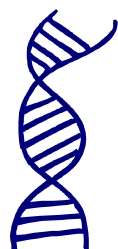
Molecular Laboratory Procedures for Detection and Characterization of Etiologic Agents

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Each year, the Indiana State Department of Health (ISDH) investigates several outbreaks or clusters of foodborne disease. These outbreaks often result from exposure to a common etiologic agent. Based on the epidemiological investigation, the laboratory can provide valuable testing of the implicated food and clinical specimens to detect the specific agent. With the appropriate specimen collection along with rapid transportation, the laboratory uses several techniques for detecting infectious agents. The classical methods of culturing microorganisms (bacteria, mycobacteria, fungi) using selective and differential media have been supplemented with molecular methods to detect viral agents and further characterize bacteria. Molecular methods such as Polymerase Chain Reaction (PCR) and Pulsed Field Gel Electrophoresis (PFGE) will be discussed in this article.

In 1972, the “Norwalk virus” was discovered in fecal specimens collected during the investigation of an outbreak of gastroenteritis at an elementary school. The role of caliciviruses (previously classified as the Norwalk family of viruses) as important pathogens remained stalled for many years until newer methods were developed and implemented. The virus was rarely seen by electron microscopy (EM) and could not be amplified in cell culture or animal models. Many foodborne outbreaks were labeled as “probably viral”. Lacking a simple sensitive assay, the role of this viral agent was unknown.



This situation improved dramatically in 1995 when the reverse transcription-polymerase chain (RT-PCR) reaction assay became the laboratory standard. RT-PCR combines two molecular techniques, reverse transcription and polymerase chain reaction to generate and amplify DNA copies from RNA transcripts. A small fragment of a specific nucleic acid sequence is amplified rapidly and exponentially using unique primers, then synthesis of a complete nucleic acid sequence (polymerization) occurs during a rapid self-contained chain reaction inside a programmable thermocycler. Within a few hours, the amplified product can be visualized and sized directly on an agarose or polyacrylamide gel.



In 1993, a large foodborne outbreak caused by *Escherichia coli* O157:H7 occurred in the western United States. The Centers for Disease Control and Prevention (CDC) scientists performed DNA “fingerprinting” by PFGE and determined that the strain of *Escherichia coli* found in hamburger patties served at a large chain of regional fast food restaurants. Because this outbreak and its cause were recognized quickly, the ground beef patties were recalled, and an estimated 800 infections were prevented. At that time, few state public health laboratories performed PFGE fingerprinting. Because the PFGE had such an important role in this investigation and state health departments had increasing demands for DNA fingerprinting, CDC developed standardized PFGE methods so that patterns from different laboratories could be generated and compared accurately.



Since then, several local and state health departments have gained more experience using PFGE of *Escherichia coli* O157:H7 to identify and control the source of the infection in several outbreaks.

When a bacterial cell divides, the two daughter cells have the same genetic makeup as the parent cell. In epidemiological terms, these bacteria are clones or clonally related; they have a common origin or parent. Even after many generations, bacteria descended from the same parent will have virtually identical genetic material, or DNA. The DNA “fingerprinting” by PFGE is a simple way of comparing genetic material between cells. This involves cutting up the DNA into pieces. The pieces are separated by a gel, which acts like a sieve. The DNA that has been cut in pieces is placed at one end of the gel. A pulsing electric field applied across the gel moves the DNA pieces across the gel over a period of hours. The smallest pieces move quickly through the gel and the larger pieces move slower. The pieces are separated as distinct bands on the gel. This pattern of bands, which resembles a bar code, is the fingerprint. Using an image-capture system and computer software, PFGE patterns are captured and entered into a database of DNA fingerprints.

Patterns can be sent by e-mail to other laboratories and CDC and are then compared to the “Hot List” of current PFGE patterns under investigation in other parts of the country and placed into a national database. In collaboration with the Association of State and Territorial Public Health Laboratory Directors, CDC created PulseNet, a nationwide electronic surveillance system so that scientists at public health laboratories throughout the country could rapidly compare the PFGE patterns of bacteria isolated from ill persons and determine whether they are similar. Similar PFGE patterns suggest that the bacteria isolated from ill persons come from a common source; for example, a widely distributed contaminated food product. Strains isolated from food products by regulatory agencies can also be compared with the isolates from ill persons. Identifying these connections can help to detect outbreaks and remove contaminated foods from the marketplace.

Today’s public health professionals are fortunate to have a variety of tools to detect viral agents and characterize bacterial isolates. In 2000, with the assistance from CDC and the Minnesota State Department of Health, the ISDH Laboratories have adopted the PCR method for calicivirus detection and PFGE analysis of *E. coli* O157:H7. The use of new technology will play an important role in surveillance and investigating of foodborne outbreaks that were previously difficult to detect.

Specimen Submission Guidelines for Molecular Testing

For PFGE Testing

Pure isolates of *E. coli* O157:H7 can be submitted using the ISDH #10A Reference Culture Kit or other approved mailing container. Each #10A kit contains an Enteric Reference Culture request form and instruction sheet (State Form 25601), absorbent packing material and shipping containers. Please submit an 18-24 hour pure single colony culture transfer on a general nutrient or infusion agar slant. Please do not submit isolates in broths, agar plates, or triple sugar agar slants. *E. coli* O157:H7 isolates are examined for O157 somatic and H7 antigens.

The request form should include the patient's name, age, sex, address, specimen source, data of onset, and specimen collection date. The person submitting the form should include the full address along with a contact person and telephone number. This is helpful when additional information is needed, and it will expedite reporting of test results.

Containers may be obtained by calling (317) 233-8104, or writing to:

Container Section
ISDH Laboratories
635 N. Barnhill Drive, Room #2031
PO Box 7202
Indianapolis, IN 46207-7202

For additional information or if you have questions, please call the ISDH Enterics Laboratory at (317) 233-8045, or e-mail Brent Barrett at bbarrett@labs.isdh.state.in.us.

For Viral PCR Testing

Testing is available as part of outbreak investigation and by prior arrangement with the ISDH. Stool specimens should be collected as soon as possible after illness onset, preferably within 48-72 hours using the ISDH #7A enteric container. Collection instructions are found on the reverse of the enteric form. Specimens should be immediately refrigerated, but NOT FROZEN. Place in an insulated box with wet ice or frozen refrigerant packs and send by overnight mail or bring by car to the ISDH Laboratories.

In addition to the 7A enteric form, complete the ISDH Virology Request Form and include it with the specimens. Both request forms should include the patient's name, age, sex, address, specimen source, data of onset, and specimen collection date. The person submitting the form should include the full address along with a contact person and telephone number. This is helpful when additional information is needed, and it will expedite reporting of test results. To obtain Virology forms or additional information, call the ISDH Virology Laboratory at (317) 233-8050 or 233-8065.

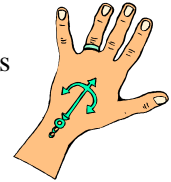
New Rule Regulates Tattoo Parlors and Body Piercing Facilities

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The 1999 General Assembly passed legislation (Senate Enrolled Act No. 38) requiring the Indiana State Department of Health (ISDH) to adopt reasonable rules to regulate the sanitary operations of body piercing facilities. This law also requires that a person under 18 years of age receiving a tattoo or undergoing body piercing must have a parent or legal guardian present and that person must provide written permission from the parent or legal guardian. Tattooing or piercing minors without the presence of, the written permission from a parent or legal guardian constitutes a Class A misdemeanor, and inquiries should be directed to the county prosecutor.



The ISDH amended the rule regulating tattoo parlors to include requirements needed for sanitation in body piercing facilities because tattooing and body piercing are frequently performed in the same facilities, and similar sanitary measures must be taken to prevent disease transmission. The rule only pertains to facilities that perform tattooing and/or body piercing as defined by the legislature. The definition of body piercing is *the perforation of any human body part other than an earlobe for the purpose of inserting jewelry or other decoration or for some other nonmedical purpose*. Thus, this law does not regulate persons that pierce the earlobe only. The rule has been signed by Governor O'Bannon and will become law July 31, 2000.



Some of the changes made in the rule include the following:

- definition changes and additions
- patron records to include type of jewelry used
- changes in disinfection and sterilization of reusable contaminated equipment
- the use of piercing guns

Definition Changes

Several changes were made in the definition sections of the rule. Tattoo parlors and body piercing facilities are both termed as a "facility". Whenever the term facility is used, the requirements apply to both tattooing and body piercing. Changes in disinfection requirements for contaminated reusable equipment made it necessary to add definitions for both "high level disinfection" and "intermediate level disinfection".

Patron Records

Patron records shall be maintained by the operator of a facility for two (2) years and shall include the type of jewelry or other decoration used. It was necessary to make changes to the information kept on the record so that the record would accurately reflect what procedure was performed.

Disinfection and Sterilization of Reusable Contaminated Equipment

The Centers for Disease Control and Prevention (CDC) has developed general principles for determining the level of disinfection or sterilization for medical equipment. The ISDH used this information as a guide when developing disinfection and sterilization requirements of the rule. Information on the sterilization/disinfection principles is available for review at the following Internet address:

<http://www.cdc.gov/ncidod/hip/Sterile/Sterilgp.htm>

The following were added to the “reusable equipment” requirements of the rule:

- Reusable contaminated equipment shall be effectively cleaned prior to sterilization or disinfection.
- Any reusable contaminated equipment that comes into direct contact, or is likely to come into direct contact, with an instrument that penetrates the skin other than a piercing gun shall be effectively cleaned and sterilized prior to use.
- All sterilized equipment shall not be removed from wrappers or sterilizer packaging until immediately prior to use.
- Any reusable equipment that comes into contact with mucus membranes shall be effectively cleaned and sterilized prior to use.
- Piercing guns shall be cleaned and undergo, at a minimum, high level disinfection after each use and whenever visibly contaminated.
- All reusable equipment that has contact with intact skin shall undergo, at a minimum, intermediate level disinfection.
- All other equipment used during the tattooing or body piercing procedure shall be single use, including corks.
- All body piercers and tattoo artists shall comply with all other equipment manufacturer’s recommendations.

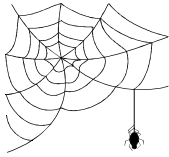
Instead of listing disinfection and sterilization requirements for each piece of equipment used in body piercing and tattooing, equipment is sterilized according to the way in which it comes into contact with the body.

Piercing Guns

Under this rule, piercing guns shall be cleaned and undergo at, a minimum, high-level disinfection after each use and whenever visibly contaminated. This rule does not apply to piercing of the earlobe. Thus, individuals piercing earlobes only would not be subject to this rule.

The ISDH is currently updating information to share with the tattoo artists and body piercers. Since many aspects of the tattoo rule of 1998, such as requiring training on bloodborne pathogens and infectious waste handling, are included in this rule, the updated information will be similar to the current information provided for tattoo artists. A new Patrons Rights document is being created, as well as other documents to educate the tattoo artists and body piercers.





Wonderful Wide Web Sites

ISDH Data Reports Available

The ISDH Epidemiology Resource Center has the following data reports and the Indiana Epidemiology Newsletter available on the ISDH Web Page:

<http://www.state.in.us/isdh/> (under Data and Statistics)

Indiana Cancer Incidence Report (1990, 95)	Indiana Mortality Report (1995, 97)
Indiana Cancer Mortality Report (1990-1994)	Indiana Natality Report (1995, 96, 97)
Indiana Health Behavior Risk Factors (1995-96, 97, 98)	Indiana Natality/Induced Termination of Pregnancy/Marriage Report (1998)
Indiana Hospital Consumer Guide (1996)	Indiana Report of Diseases of Public Health Interest (1997, 98)
Indiana Marriage Report (1995, 96, 97)	

The following site allows access to the web page for any state health department in the United States:

<http://www.polsci.wvu.edu/grad/klase/STATEHEALTH/sthlth.html>

HIV Disease Summary

Information as of June 30, 2000 (population 5,840,528).

HIV - without AIDS to date:

233	New cases from May 1999 thru June 2000	12-month incidence:	3.99 cases/100,000
3,163	Total HIV-positive, without AIDS on June 30, 2000 ¹	Point prevalence:	54.16 cases/100,000 ¹

AIDS cases to date:

324	New AIDS cases from May 1999 thru June 2000	12-month incidence:	5.55 cases/100,000
2,548	Total AIDS cases on June 30, 2000 ¹	Point prevalence:	43.63cases/100,000 ¹
5,895	Total AIDS cases, cumulative (alive and dead)		

¹ Counting only cases alive in June 2000

REPORTED CASES of selected notifiable diseases

Disease	Cases Reported in June		Cumulative Cases Reported through June	
	1999	2000	1999	2000
Campylobacteriosis	48	69	188	182
<i>E. coli</i> O157:H7	2	12	16	25
Giardiasis	38	41	191	215
Hepatitis A	8	5	65	30
Hepatitis B	4	6	25	26
Legionellosis	7	7	20	16
Lyme Disease	11	4	16	7
Meningococcal, invasive	10	1	32	27
Pertussis	4	5	14	27
Rocky Mountain Spotted Fever	0	0	2	0
Salmonellosis	36	63	184	256
Shigellosis	19	394	53	760
Tuberculosis	8	12	60	66
Animal Rabies	0	0	0	0
For information on reporting of communicable diseases in Indiana, call the <i>ISDH Communicable Disease Division</i> at (317) 233-7665.				

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Newsletter

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